ligation of ssDNA

ligation of duplex DN

~ (SEQIDNO:12) TTCACGAGCCTG

GATCAGGT

CT AGT CCAAAGT GCT CGG (SEQ &D NO. 13)

(SEQIO NO(15) TGTACGCT GGAT GCA TTCAT GCG ACCT ACGTAGGT CGCAT G T <sup>I</sup> (SEQ ID NO!15)

GATCAGGT. TT CACGAGCCTG (SEQ ED NO', 14)

TTGTACGCTGGATGCATCCAGCGTACT TOAT GCGACCT ACGT AGGT CGCAT GT T ( SEQ ID NO! 16)

one pot ligation / cyclization of ssDNA

(SEQID NO:17)

GTTTT A TACAAAACCT GGCA

TT CAGCAAATAT GT<sup>, S - I</sup>TTT G(IACCGTT GGT<sub>T</sub> r(seq 20 NOL 19) (SEQ 20 NO:18)

> TCAGGAGACTGTTCAG CT GCT TCACT AGT.

> > AGT GAT CAAGT CCT CT GA

TT CAGCAAATAT GT.TTTGGACCGTTGGTT (SEQ EU NO(21) C CT GCT TCACT AGT. TCA GGAGACT GT TCAG

isolated vield conversion rxn. type 44% >90% ssDNA ligation 36% 75% duplex ligation >90% (1st step) ligation/cyclization 20% 50% (2nd step)



## exonuclease / hydrolysis susceptibility

5-GATCAGGTp.TTCACGAGCCTG.3 (SEQ 2D NO:14)

endonuclease susceptibility

TTGTACGCTGGATGCARTCCAGCGTACTT (SEQ #0 /VD://6)

template for replication / transcription

(SEQ ED NO! 22)

\*ATTAT GCT GAGT GAT ATCCTGCCTATTCCGAGCACTTp,T GGACTAG



dumbbel!

5' bridging phosphorothloate duplex:

) TTGTACGCTGGATGCASTCCAGCGTATCTT
TCATGCGACCTSACGTAGGTCGCATAGTT

all phosphodiester duplex:

Nail

TTGTACGCTGGATGCATCCAGCGTATCTT
TCATGCGACCTACGTAGGTCGCATAGTT

hairpin



(≤€9≠0N0(27) MUT target 3 CCACCACCGAGGCCGCCACACCCATTC5 (SEQIDNC 28) 5'MM target (SEQ EO NO. 29) 3'MM target \*CCACCACCGAGGAAGCCACACCCATTC\* (SEQ DONC', 30) MMM target \*CCACCACCGAGGCAGGCACACCCATTC (SEQ 10 NO: 31) cyclization probe (SEQ EONO: 27) MUT target #CCACCACCGAGGCAGCCACACCCATTC# (SEQ 20 No. 28) 5'MM target \*CCACCACCGAGGCCGCCACACCCATTCs (SEQ ID NO: 29) 3'MM target 3 CCACCACCGAGGAGCCACACCCATTC5 CCACCACCGAGGCAGCCAAACCCATTCs (SEQ ID NO 32) MMM target

(SEQ ID NO. 34) Syclization probe

GCGCCGp. TCGGTGTGGGTTTT

(SEQ ID NO. 33) MUT target FACTACGCGGCAGCCACACCCAAAAGTTCF

(SEQ ID NO. 34) 3'MM target FACTACGCGGAAGCCACACCCAAAAGTTCF

(SEQ ID NO. 35) MMM target FACTACGCGGAAGCCACACCCAAAAGTTCF

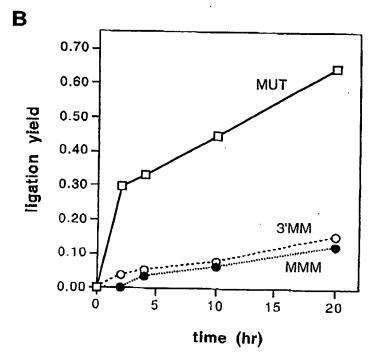


Fig. 11

A

| SEQ #0 NO; 36|
| TGAGAACGGGTGT: MGGCTGCC

(SEQ #0 NO; 37) WT HITGEL FGTCAGCGCACTCTTGCCCACACCGCCGG—

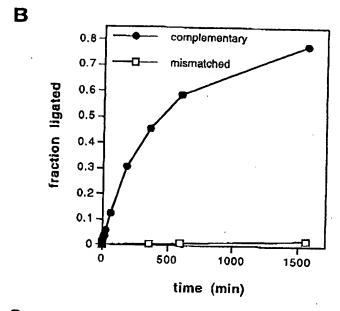
| CGCCCACCACCACCACCACCGCCGCG—

| CGCCCACCACCACCACCACCGACGG—

| CGCCCACCACCACCACCACCGACGG—

| CGCCCACCACCACCACCACCACCGACGG—

| CGCCCACCACCACCACCACCACCTTATAP



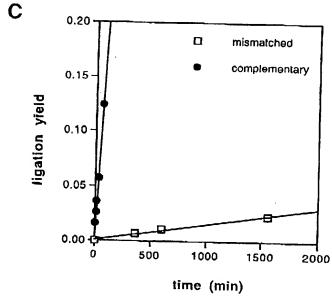


Fig. 12

probes TGAGAACGGGTGT: -GGCXGCC (X=G,T)

WT target

SGTCAGCGCACTCTTGCCCACACCGCCGGCGCCCCACCACCAGCTTATAGATTAG

MUT target

5'GTCAGCGCACTCTTGCCCACACCGACGCCCCACCACCACCAGCTTATA3

(SEQ 20 NO. 38)

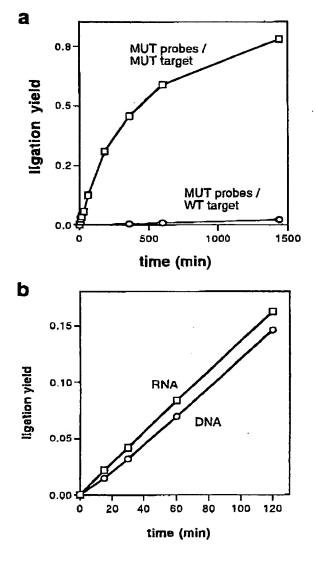


Fig. 14



## universal probe (FAM label)



probes 3, (SEQ ED NO; 36)
TGAGAACGGGTGT: SeGGCXGCC5 (X=G,T)

WT target

5'GTCAGCGCACTCTTGCCCACACCGCCGCCGCCCCACCACCACCAGCTTATA3'
(SEW ≱D Nº137)

MUT target (SEQ DO NO(38)

5'GTCAGCGCACTCTTGCCCACACCGACGCCCCACCACCACCAGCTTATA3'

MUT RNA target (SEQ ID NO! 40)

5'GCGCACUCUUGCCCACACCGACGCGCCC3'

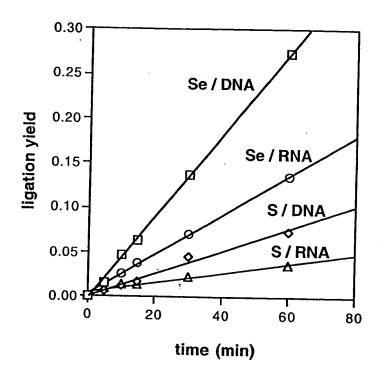


Fig. 20